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SOIL MICROBIOLOGY AND ITS CURRENT PROBLEMS

by Ye. N. Mishustin

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Ye.N.Mishustin

Problems in the afforestation of grassland and wasteland are discussed from the viewpoint of soil microbiology, including aspects of increase in grain and other harvests by optimum soil management. Symbiotic association of fungi and higher organisms is suggested for growth promotion of young tree plantings, with such soil to be provided by "mycorhiza nurseries". Grassland conservation cropping and artificial humus enrichment by microbe inoculation and suppression of aerobic bacteria are discussed in their theoretical and practical aspects. The Waksman theory of humus formation in the soil by polymerization of lignin and bacterial proteins is disputed, and the theory of combination of protein matter with urononic acids from pectin decomposition is advanced instead. A brief review of microbiological soil testing is included, with tabulated data on microbe contents of various types of soil.

1. Introduction

The realization of the historic decree of the USSR Council of Ministers and of the Central Committee of the USSR Communist Party on the plan of protective afforestation and the introduction of grassland crop rotation places on Soviet scientists, and in particular on the representatives of agricultural microbiology, the obligation of answering in the near future a number of extremely important questions of agronomy. In this connection it will be necessary not only to put into actual production the accomplishments of agricultural microbiology, but also to place on the agenda for a speedy solution a number of new problems that will arise during the fulfillment of this magnificent plan.

In speaking of the soil and the factors determining its fertility, it is appropriate to recall that the biological trend is characteristic for Russian agronomy. The immense significance of living organisms to the formation of soil was noted already by that great Russian scientist, M.V.Lomonosov. His views on agronomy determined the course of science in other countries for many decades, although the soil had long been regarded as a purely geological formation.

The traditions inherent in Russian agronomy can be conceived as continued and preserved in the fact that this branch of knowledge in the USSR forms part of the biological disciplines.

After the famous work of L. Pasteur, who defined the role of microorganisms

^{*} Numbers in the margin indicate pagination in the original foreign text.

in the transformations of organic and mineral substances, it became common knowledge that an immense number of microorganisms exist in the soil.

The founders of scientific agronomy, including V.V.Dokuchayev, N.A.Kosty-chev and V.R.Vil'yams, viewed it as obvious that bacteria play an important role in soil processes and that their activity must substantially influence soil fertility.

V.V.Dokuchayev (1846-1903), in developing the scientific foundations of modern agronomy, made every effort to promote the study of the microcosmos. He persistently attempted to organize departments of microbiology at the universities, but he was unsuccessful under the conditions of the Czarist regime.

It should be noted that one of Dokuchayev's ideas was to create protective forest belts in the steppe and wooded-steppe regions for the purpose of drought control.

N.A.Kostychev (1845-1895) made a number of studies on the microbiological processes taking place during the decomposition of plant residues and the /156 formation of humus. He attached exceptional importance to microbiological processes in soil formation. Speaking of the origin of the chernozem, he wrote: "Geology ... is of secondary importance in the chernozem question, since the accumulation of organic matter takes place in the upper layers of soil that are geologically different, but the chernozem is ruled by the geography of the higher plants and by the physiology of the lower plants which cause the decomposition of organic matter" (Bibl.21, p.8).

Specifically, in that paper, Kostychev developed a theory of drought control by correct soil management and by snow retention.

Academician V.R.Vil'yams (1863-1939) played a major role in modern agronomy and placed this science on the foundation of the Marxist doctrine of soil fertility. He developed in detail the biological aspect of the problem of the origin of soil and formulated the doctrine of phytogenesis. He considered that certain groups of higher green plants and lower nonchlorophyll plants belonged to different periods of the same process of soil formation.

According to this author, the principal natural combinations of chlorophyll and nonchlorophyll plants, which he called plant formations, can be divided into the following groups:

- l. Forest plant formations, consisting of associations of woody green plants, fungi, actinomycetes, and anaerobic bacteria.
- 2. Meadow grassy plant formations, with coexistence of herbaceous plants, aerobic bacteria, and in considerable numbers representatives of the anaerobic bacteria.
- 3. Steppe grassy plant formations, consisting of steppe herbaceous plants and aerobic bacteria.
 - 4. Desert plant formations, including chemotrophic bacteria and algae,

aerobic and anaerobic bacteria, and also fungi.

These concepts formed the basis for the detailed investigations by Soviet microbiologists who have the task of refining his general outlines.

Vil'yams had a high opinion of the work of Dokuchayev, Kostychev, and other classical Russian agronomists, and created an orderly, scientifically substantiated system of measures to increase the productivity of agricultural crops and animal breeding, which he termed the grassland system of agriculture. This system is called the Dokuchayev-Kostychev-Vil'yams complex in the decree of the USSR Council of Ministers and the Central Committee of the USSR Communist Party dated September 20, 1948, and entitled: "On a plan of protective afforestation, the introduction of grassland crop rotation, the construction of ponds and reservoirs to ensure high and stable yields in the steppe and wooded-steppe regions of the European part of the USSR".

In his well-known work "On the Agronomic Doctrine of V.R.Vil'yams" (Ob agronomicheskom uchenii V.R.Vil'yamsa), T.D.Lysenko writes: "The theory of V.R.Vil'yams on soil formation, on the processes of development and the impairment of the conditions of soil fertility, in which he takes a creative attitude, makes it possible in agronomy to work out measures which, as a result of the /157 biological processes in the soil and as a result of the vital processes of plants and microorganisms, will increase the soil fertility and convert infertile and even sterile soil into fertile land. The theory of Vil'yams is of acute significance, since it indicates precisely what biological and physicochemical processes will improve soil fertility and what processes affect it adversely.

"For this reason, it is entirely justified to say that the Vil'yams doctrine of the laws of soil management and of the development of its fertility constitutes the theoretical foundation for the natural control of soil fertility in agriculture" (Bibl.30).

The views of Dokuchayev, Kostychev, and Vil'yams, as the focus of a lens, concentrated the thoughts of the progressive Russian scientists who lived and worked in the second half of the last century, when the interest in microorganisms and in defining their role in nature was particularly intense.

We recall that the symbiosis of leguminous plants with Rhizobia was discovered by M.S. Voronin and was studied by L.S. Kossovich (1890) and K.A. Timiryazev (1892), and by others.

In the 80's, F.M.Kamenskiy (1881) established mycotrophism in the higher plants. This phenomenon was of great interest to Kostychev (1890) and Voronin (1895), who devoted special studies to it. In the 90's, there were a number of brilliant studies by S.N.Vinogradskiy on the process of nitrogen fixation and the autotrophism of bacteria. In the same period, D.I.Ivanovskiy (1892) discovered the filtrable viruses.

Toward the 90's, there were a number of public addresses by famous experts of Russian agronomy, emphasizing the role of microorganisms in soil processes. Two papers specified to this field were read before the Eighth Congress of

Natural Scientists and Physicians at St.Petersburg (1890). V.I.Kovalevskiy presented a paper "The Demands of Modern Agriculture on Science". He mapped out an extensive program of research work in the field of agronomic biology. Among other things, he stated that the soil is a center of life and struggle for existence of microscopic plant organisms and that the productivity of higher crop plants is predetermined more or less by these microorganisms, which in turn demand proper cultural methods.

At the same Congress, Professor G.G.Gustavson, of the Petrovskoy Agricultural Academy, presented a paper "The Microbiological Foundation of Agronomy". He showed convincingly that the soil is a living system and that lower organisms must play a major role in its properties (Bibl.12). A year later, the famous Russian scientist, D.I.Ivanovskiy, published a work "The Activity of Microorganisms in the Soil", prefaced by a statement by Academician A.S.Famintsyn:
"It seems justified to assume that, at some future date, successful cultivation and increase in harvest of grain will be primarily determined by adaptation of the soil to an abundant growth of microorganisms" (Bibl.15).

The reader will involuntarily compare these words with Viltyams remark that, after the outstanding work done by Pasteur, Mechnikov, Bakh, Vinogradskiy, Kholodniy and many others, the soil can no longer be considered as a dead substrate. If we were to adopt such a view, then this "... would mean to dis- /158 believe in the progressive development of science, to disbelieve in the progress of social and productive relationships. It would mean being half a century behind real life" (Bibl.5, p.112).

From the above quotations, it becomes obvious that 60 years ago the scientists and progressive agronomists were already quite clear as to the significance of the micropopulation in determining soil fertility.

In referring to the problems of agricultural science, Vil'yams wrote: "The central question of agronomical science is the struggle for soil fertility" (Bibl.5, p.8). All his activity was devoted to this problem, and in solving it he closely related theoretical development and socialist practice.

Lysenko remarked: "The Michurin doctrine and the Vil'yams doctrine, which merely represent different aspects of the same materialistic biological science but were not recognized by the obsolete idealistic biology, have become, under the conditions of socialist agriculture, the biological foundation of the science of agronomy, merging into a single agrobiological discipline" (Bibl.31).

Vil'yams asserted that, at the present level of knowledge, the most perfected system of agriculture is the grassland system which ensures simultaneous development of the most important branches of agriculturel production, plant growing, and animal husbandry.

As indicated in the historic decree of the Party and Government of October 20, 1948, the essence of the Dokuchayev-Kostychev-Vil†yams complex is the inseparable realization of the following agronomic measures:

a) Planting of protective belts along watersheds, along the boundaries of the rotation fields, on the slopes of ridges and ravines, on the

shores of rivers and lakes, around ponds and reservoirs, and also the afforestation and fixation of sands.

- b) Proper management of the land area, with the introduction of grassland field and forage crop rotation and rational utilization of the land plots.
- c) Correct system of cultivation and care of seeding, with emphasis on plowing by colter plows, widespread use of black fallow, plowland and stubble plowing.
- d) Correct system of application of organic and mineral fertilizers;
- e) Sowing selected seed of high-yield varieties adapted to the local conditions.
- f) Development of irrigation by all possible means based on utilization of the local runoff waters by construction of ponds and reservoirs.

Agricultural microbiologists can in many respects cooperate in the realization of these measures, based on the valuable data accumulated by earlier generations of scientists, and in solving those questions that had not been fully settled previously. Below, we will draw attention to a series of actual problems whose immediate solution, in our opinion, is dictated by the present situation.

2. Planting of Protective Forest Belts and Soil Microbiology

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The existence of symbiotic relations between higher plants and fungi was first established by the Russian scientist F.M. Kamenskiy (1881). It was later demonstrated that a considerable number of trees belong to the superfamily of mycotrophic plants and develop less well in the absence of suitable soil fungi which, together with the root system, represent a symbiotic formation known as "mycorhiza". An analysis of the interrelations of the symbiotic association of fungi mycelia with the higher plants has recently been given in a number of papers (Mishustin, Lobanov, Krasovskaya, and others) so that we need only discuss a few basic problems.

At present, it can be considered proved that symbiotic fungi promote the assimilation of nutrients and water from the soil by the higher plants. This function is the result of an increase in the adsorptive surface of the rootwork and of the capability of fungi to transform soil substances, unavailable to the plant, into readily assimilated compounds.

Mycotrophism is manifested in varying degrees by different plants. In accordance with this criterion, N.V.Lobanov divided the plants into the following four groups:

1. Obligate mycotrophic plants, which cannot develop without the presence of fungi. This group includes only a few herbaceous plants.

- 2. Plants with pronounced mycotrophism. This group includes several trees, such as oaks, hornbeams, beeches, conifers, etc.
- 3. Plants able to develop without mycorhiza. Among the trees and bushes, these include birch, acacia, and fruit trees.
- 4. Plants intermediate between Types 2 and 3.

It is of great importance for farming practice that the composition of the mycorhiza is quite specific among the various trees. Very rarely, however, will a mycorhiza exist in the same plant with a number of different fungi, and it must be assumed that the growth is far from indifferent to the species of fungus with which the plant is in symbiotic association.

The mycorhiza-forming fungi belong mostly to the class of Basidiomycetes, among which species of the genus Hymenomycetes predominate. Species of the division of Ascomycetes, Phycomycetes, and of the group of Fungi imperfecti are more rarely encountered.

Most of such fungi grow poorly and slowly on the usual nutrient media. This fact makes their isolation and the study of pure cultures difficult, and also interferes with the utilization of such pure cultures for inoculating young plantings and seedlings. For such purposes, a soil from suitable forest plantings ("mycorhizal land"), with an already developed complex of useful microorganisms, is ordinarily utilized.

Studies by our Laboratory have shown that a highly specific bacterial flora develops in the rootwork of trees. There is reason to believe that it likewise exerts an influence on the development of the roots.

Today, thanks to the work by G.N.Vysotskiy (1902-1929), N.A.Yurre (1939), A.V.Baraney (1940) and others, it may be considered proved that the use of mycorhizal land in setting out young trees on soils that were not previously /160 wooded, is entirely justified, since it ensures better development of the seedlings and transplants.

Realization of the ingenious plan of protective afforestation will confront microbiologists with a number of new questions which must be solved in the shortest possible time. The following are the principal such problems:

- a) Definition of type of soils in which mycorhiza-forming fungi are encountered and soils from which they are absent. This will permit a closer delimitation of the territory subject to obligate mycorhization.
- b) The removal of mycorhizal soil from forest areas leads to the inevitable loss of the root systems of the trees. Thus, possible replacement of such soils by soils artificially enriched with mycorhiza-forming fungi is in question.
- c) Possible inoculation at the storage sheds where the planting material is kept, instead of in the field, which leads to considerable compli-

cation in planting.

- d) Instructions for preparation of mycorhizal soils. In particular, doing this ahead of time, for instance in autumn. There is also the question of the development of the scientific bases of the dosage of mycorhizal soil.
- e) Definition of the fertilizing system that will promote optimum mycorhiza formation and thus optimum growth of the young plants.
- f) Improving the species of fungi that form mycorhiza with the principal species of trees and possible use of pure cultures of such organisms in agricultural practice.
- g) Definition of the physiological principles of interrelation between the higher plants and the fungus symbionts.

The trend of this work was delimited by the results of conferences held at the Central Administration of Shelter-Belt Afforestation, USSR Ministry of Forests.

The solution of the first problem is directly connected with an investigation of the features of the microbial association within which the mycorhizaforming fungi usually exist. Recent work by our Laboratory has shown the microflora of forest soils to be entirely specific. It is also well known that Basidiomycetes, among which many mycorhiza formers are encountered, develop only in forest plantings in symbiotic association with higher plants. It is therefore natural that in soils with meadow or steppe vegetation, the formation of mycorhiza is not very satisfactory and is slow, unless artificial inoculation is used. The only exception is constituted by soils that had been covered by forests in the relatively recent past. There is a distinct need of refining the ecology of mycorhizal fungi, and it is well possible that some soils should be removed from the list of those obligatorily to be infected during afforestation. It must be remembered that for each hectare of new planting, as much as 500 kg of mycorhizal soil must be brought in; this work should be avoided wherever possible. Under steppe and meadow formations, as we have shown, microbial associations that inhibit the growth of fungi are occasionally encountered. This is entirely natural, since in such cases we are dealing with a dominant multiplication of bacteria in the soil, many of which have strong antagonistic properties. It is therefore necessary not only to think of inoculating forest plantings with fungi but also of establishing, in the soil, conditions favoring the growth of the introduced organisms. This may be accomplished by the use /161 of proper fertilizer, certain planting schedules, etc.

The question of proportional ratios of mycorhizal soil addition has not yet been reliably answered. It is well possible that the proportions used until now will have to be considerably modified, depending on the type of the original soil and the properties of the mycorhizal soil.

It would be desirable to attempt an inoculation of seeds by mycorhizal soil already in the storehouse. For example, when storing acorns for the winter, they could be sprinkled with earth taken from under oak plantings. If this

measure proved justified, the tedious work of bringing in mycorhizal soil in Spring would become unnecessary. Certain observations by Vysotskiy seem to indicate that this question may be answered in the affirmative.

The question of a completely equivalent exchange of soil taken from existing forest plantings is gaining in importance. It would be advisable to use pure cultures of mycorhizal fungi, but there are still several difficulties involved. For example, the symbiotic fungi of most trees are isolated with great trouble, and their pure cultures grow with extreme slowness on artificial media. In the near future, this fact will limit the use of pure fungal cultures in afforestation. Of course, work in this direction should continue, the more so since, according to preliminary reports, several investigators have succeeded in isolating pure cultures of such fungi and have submitted them for production trial runs (K.I.Rudakov, Ya.N.Khudyakov, N.M.Shemakhanova, et al.).

As already stated, a specific microflora multiplies in the rhizosphere of trees. It might well be that the introduction of preparations of certain bacteria into the seeds of the trees will promote the development of the young trees.

The proposal by N.A.Yurre to use the soil of greenhouses for inoculating young plantings with mycorhizal fungi seems highly useful. F.Yu.Gel'tser, in further developing this suggestion, recommended the creation of "mycorhiza nurseries". The latter term would mean forest plantings on well-cultivated subsoils. In the soil of such nurseries mycorhizal fungi would multiply, making it useful for replacing mycorhizal earth. Such "mycorhiza nurseries" would eliminate the risk of damage to existing forest plantings by removing their "mycorhizal soil". Fomin is working along the same line.

As already noted, we must solve the question whether advance preparation of "mycorhizal earth" is possible. Today such earth is taken from the spring forest, immediately before planting the forest belts. This causes great pressure in the work and makes it difficult to transport the loads on the muddy roads. If the mycelia of the fungus could favorably winter in the soil, delivered to the site of future use in the autumn, it would be preferable to bring in the "mycorhizal earth" ahead of time.

Finally, we must solve the question of the fertilizing system to be used for the young tree plantings so as to promote the formation of mycorhiza.

Judging from the experiments in our laboratory, and in other Research Institutes, phosphate and organic fertilizers have a particularly favorable effect on mycorhiza formation. Mineral nitrogenous fertilizers usually have a pronounced retarding effect on the development of mycorhiza. This can be attributed to the fact that the protein synthesis of the plant is intensified in /162 the presence of excess nitrogen. This leads to a depletion of the root system in carbohydrates. The carbohydrate deficit interferes with the normal interrelations between higher and lower organisms.

It may be stated that entirely analogous phenomena are observed in leguminous plants. Their fertilization by mineral nitrogen retards the nodule development.

It must be recognized that the intimate connection between the higher organism and the fungus symbiont has been insufficiently studied, a gap that must be filled.

In large-scale forest plantings, acacia and other trees (for example, oleaster) that live in symbiotic association with bacteria, should preferably be used. The effectiveness of their inoculation with suitable agents should be studied and, if necessary, useful bacterial preparations for production should be provided.

Introduction of Grassland Complication and Problems of Microbiology

V.R. Vil yams developed a doctrine of grasslands agronomy, comprising a theoretical substantiation of the assumption that not only high and stable harvests but also record harvests can be obtained at present.

"Despite the positive and progressive theoretical foundation of grasslands farming, it must be pointed out that Vil'yams' grassland agronomy doctrine contains a number of erroneous premises, which must be criticized in the interest of science and practice" (Bibl.30). These points were analyzed by T.D.Lysenko.

Below, we will specifically discuss several of these propositions.

Vil'yams attached great importance to the structure of the soil which, under production conditions, is formed by the growing of perennial grasses. The usefulness of perennial grasses as conservation cropping in field crop rotation, to improve the yield of all crops and to provide satisfactory forage for animal husbandry, has been proved beyond all doubt.

Vil'yams correctly pointed out that the most favorable air-water regime is established in structured soils, and that inorganic fertilizers work most effectively there.

"A structured soil frees mankind's most important industry, namely, agriculture, from its direct dependence on the annual rainfall", wrote Vil'yams (Bibl.5, p.3).

The soil acquires an agronomically valuable structure as a result of a cementation of its particles by the active humus, formed by certain groups of microorganisms from decomposed organic matter.

It should be noted that, in speaking of the conversion of organic matter in the soil, Vil'yams took a very broad approach to this question. He analyzed the possible modes of conversion of plant residues in the soil, and, while placing special emphasis on the activity of microbes, did not lose sight of the purely chemical processes present. He wrote: "All that we have said does not deny the possibility of a purely mineral (abiotic) decomposition of organic matter ... /163 forces us to assume also the significance of mineral decomposition of organic matter on the earth's surface, without the aid of microorganisms" (Bibl.4, p.230).

Naturally, microbiological processes play the primary role in the formation of soil structure.

According to the Vil'yams doctrine, the stand of a grass field should consist of equal numbers of stalks of perennial grasses and leguminous plants. The biological features of perennial grasses involve the decomposition of their residues during the period of domination of anaerobic processes, capable of producing active humus (ulminic acid and ulmin). This latter has the properties of an enzyme and imparts structure to the soil particles. Decomposition of the roots of the leguminous plants furnishes considerable quantities of calcium salts thus giving strength to the crumbs thus formed.

The humic substances may likewise be formed by aerobic bacteria (humic acid) and by fungi (crenic acid), but these substances are unable to form a strong soil structure. On exposure to air, they are rapidly decomposed by aerobic microorganisms.

The basic principles of the Vil'yams doctrine on the structuring of the soil and on the humic substances as products of microbial synthesis may be considered confirmed. However, many major problems on the process of structure formation have been insufficiently investigated. The most important of these questions relate to the sources of raw material for humus formation, and to the mode of synthesis of the humic substances which are high polymers.

Not so long ago, the Waksman theory was widely accepted. According to this theory, the humus in the soil is formed mainly by polymerization of lignin and bacterial proteins. Other substances were rejected as sources of humus, in view of their rapid decomposition by soil microflora.

The Waksman hypothesis does not correspond to reality. First of all, humus formed from lignin cannot possess cementing properties, i.e., it cannot be the "active humus" that imparts a strong structure to the soil. In addition, the main "nucleus" of humus compounds has a structure differing from that of lignin, and lignin must be fundamentally reorganized by the microorganisms before humus can be synthesized from it.

Compounds of the type of ulmic acid should of course be formed in a different way, which has not yet been experimentally defined. The hypotheses advanced by individual investigators are not sufficiently substantiated. Nevertheless, they do disclose conceivable modes of formation of active humus. Thus, K.I. Rudakov suggested that active humus is formed by a combination of protein matter and urononic acids formed on the decomposition of pectin.

M.M.Kononova attaches great importance in this process to cellulosolytic myxobacteria and to the considerable quantity of microbial plasma that accumulates in the medium. The interaction of the element of the plasmodium of myxobacteria with soluble polyphenols (tannin substances, polyphenols of secondary origin, etc.) leads to the formation of humic acid. This interaction, in /16/4 Kononova's opinion, must be accompanied by oxidative processes induced by the ferments of the cellulose myxobacteria.

F.Yu.Gel'tser believes that a strong soil structure is formed by cementa-

tion with the plasma of dead bacteria. This hypothesis obviously leads to the assertion that the cell contents of the microorganisms are equivalent to humus.

Our own experimental study (Mishustin and Gromyko) indicates that the mycelia of fungi and actinomycetes, as well as myxomycetes or slime molds, may aggregate the soil, but that this bonding is unstable and easily disrupted. Recently, a similar conclusion was reached by K.I.Rudakov. It should therefore be recognized that the structure of soil formed by myxomycetous organisms and fungal mycelia is fundamentally different from the structure formed by active humus.

It seems that it would be difficult to limit the list of possible producers of active humus. Apparently, depending on actual conditions, the group of organic substances converted into humus may be larger or smaller. The process involved must be considered as a reaction taking place under microaerophilic and quasi-anaerobic conditions.

The products of the vital processes of microorganisms no doubt play the main role in the formation of active humus.

It should be emphasized that most of the above views (Kononova, Rudakov, Mishustin) envisage an obligate participation in humus formation of the ectoenzymes of bacteria, which promote the reactions of synthesis. The Gelitser hypothesis completely ignores the significance of ectoenzymes of bacterial origin in the formation of humus.

It has recently become necessary to optimize the promoting processes for accumulation of active humus. Vil'yams considered that humus of agronomic value was formed only by anaerobic bacteria. Kostychev likewise emphasized that the accumulation of any substantial amounts of humus could take place only under depression of microbiological processes. From his research it is obvious that he had in mind the suppression of the activity of aerobic bacteria. The present author shares Kostychev's view. The literature contains also other opinions. Thus, it follows from a book by F.Yu.Gel'tser "The Significance of Microorganisms in the Formation of Humus and the Strength of the Soil Structure" (1940) that a strong soil structure is formed under aerobic conditions that promote the development of the common saprophytic microflora of the soil. Based on this concept, one would have to conclude that structuring of a soil is promoted by frequent cultivation (!).

All of the above quotations seem to indicate the necessity of further detailed study of the humus problem. Specifically, the chemical aspect of such work, which is not sufficiently regarded at present, should be emphasized.

Recently, under the influence of work done by S.S.Dragunov, there has been a definite shift toward the chemistry of humus substances.

In determining the merits of a given soil structure, one is usually guided by its quantitative rather than by its qualitative indices. However, Vil'yams, correctly noted that the soil structure might be weak in some instances. In particular, this is true of soils containing a small quantity of mineral and organic suspensoids,

Vil'yams writes: "Soil containing a small amount of mineral or organic /165 suspensoids may possess considerable strength, i.e., its particles may be well cemented by water-insoluble cementing substance, but since this cement is not particularly strong, such a soil will also not have particular strength. Its structure is easily obliterated by mechanical action, which simultaneously reduces its strength" (Bibl.3, p.117).

An agronomically valuable soil structure must be stable in time and must be rated not only by an overall index but also by the length of time it persists under cultivation. Some approaches to rating the stability of a soil structure, although far from perfect, have been recommended by us.

The question of the strength of the soil macrostructure is particularly important for certain types of soil. For example, a good bed with podzols or many gray soils, yields a considerable structurizing effect, but the resultant structure breaks down rapidly on the next cultivation. Microbiologists, in collaboration with agronomists, should work out the problem of possible approaches to an improvement of the useful physical properties of a soil by grass conservation cropping. This question is organically related to a regulation of the mineralization of plant residues and organic matter in the soil. Of course, a common solution for all soil-climatic zones is impossible.

It is well known that, in the opinion of Viltyams, a given field should be plowed only in autumn, so as to help maintain the soil structure. He stated that the use of autumn cereals in a crop rotation was a sign of the technical ignorance of the farm management, and oriented farmers toward the use of spring cereals instead. This was in complete agreement with his directives for fall plowing of the soil.

The above proposition, as pointed out by Academician Lysenko, is built on wrong premises. The essence of the error lies in Vil'yams erroneous concept as to the growth aspects of autumn and spring cereals under various climatic conditions.

Lysenko writes: "The mistakes of Vil'yams and many other scientists, who are in favor of exclusive fall plowing of the soil, include primarily their disregarding the fact that in many regions of the USSR climatic and economic conditions make winter sowing not only possible but also necessary, including fall plowing instead of summer plowing; secondly, these authors have lost sight of the peculiarities and difficulties of the period of introduction of grassland farming on collective farms, covering tens of millions of hectares" (Bibl.30).

It is obvious that, in the near future, microbiologists in collaboration with other experts must solve the problem of optimum time for tilling the soil under various soil and climatic conditions.

Viltyams was justified in stating that all agronomic measures must be considered in connection with the grassland system of agriculture. This should be put into practice by the use of bacterial fertilizers. Such use must allow for the dynamics of individual microorganisms in the crop rotation. For example, /166 it has been found that the titer of the Rhizobia of clover drops sharply during crop rotation on podzolic soils. This would indicate that the leguminous grasses

should be treated with "nitragin" before sowing. However, in lime podzols, the number of Rhizobia remains constant for a long time, which would mean that a bacterization of such soils may be ineffective.

The fate of the Rhizobia of the principal leguminous crops in soils of various climatic zones and crop composition has never been followed. Unfortunately, the lack of such data makes it difficult to derive a scientifically substantiated system of application of so costly a preparation as nitragin.

The situation is no better for azotogen (azotobacterin), which contains a free fixing agent for molecular nitrogen, namely, azotobacters. The features of such bacterization of agricultural crops by the above preparation in grassland farming have never been determined with any degree of accuracy.

Individual Research Institutes recently proposed new microbiological preparations for soil amelioration (phosphor-bacteria, AMB bacteria, decomposing silicates, etc.). Extensive tests on these preparations should be combined with instructions for their use in crop rotation.

We will discuss a number of basic questions connected with the use of bacterized soil-improving preparations in the next Section.

Microbiological data may be of considerable usefulness in the selection of plants for crop rotation. It must be borne in mind that N.A.Krasil'nikov clearly demonstrated the antagonistic action of the rhizosphere of certain grasses on phytopathogenic bacteria and fungi of crop plants. These data are of a certain practical value and should be utilized widely. Crop rotation is one of the effective methods of weed control by the use of phytoparasites of plant origin. The effect of the microflora of the root system of various plants should be established and, in many cases, should be taken into account when selecting the elements of crop rotation. Verticilliose and gummosis of the cotton plant, fusariosis of cereals, and other diseases of agriculture plants, can apparently be controlled by making use of microbial antagonism.

Certain observations made in the Iaboratory of N.A.Krasil'nikov seem to indicate that, in many cases, sprinkling the diseased plants with a bacterial antagonist may have a satisfactory curative effect.

The use of antibiotics for the treating of grain may also be promising.

The high yields of soils in grassland crop rotation can be attributed to a correct and strictly scientific cultivation, taking account of the action of microorganisms in the plowed soil layer. This question, which is of direct significance for practical farming but which has been little studied, will be discussed later in the text.

The logical conclusion is that the introduction of grassland rotation will confront microbiologists with numerous problems which must be solved in the near future. We also must agree fully with the opinion by M.V.Fedorov who writes:

"No other system of agronomy pays as much attention to the control of soil microbiological processes as does the grassland system; therefore, no other /167 system can ensure such a progressive increase in yield. The great advance in

this field is due primarily to thorough study of the biological life of the soil and its control by human forces, causing the microbiological processes to proceed such as to serve the ultimate ends of production, namely, the productivity increase in agriculture" (Bibl.42, p.361).

Problems of Microbiology in Regulation of Nutrition and Fertilization of Agricultural Plants

The soil is exceptionally rich in microorganisms. Their number may go as high as several thousand million per gram of soil, and the total weight of the bacteria living in the plowed layer of one hectare of land is 5 - 15 tons.

The extremely small size of microorganisms is responsible for their enormously large active surface, which amounts to 500 hectares per hectare of plowed soil. At the intense metabolism characteristic of bacteria, this surface constitutes a source that liberates a substantial number of metabolites and ectoenzymes that have a modifying effect on the composition of the environment.

TABLE 1

SUPPLY OF CERTAIN ELEMENTS IN PODZOLIC SOILS, (kg/hectare)

Compound	Total Supply in Soil	Supply in Agronomic Compounds	Supply in Bacterial Bodies	Average Dose Introduced by Fertilizers	
Nitrogen (N) Phosphorus (P ₂ O ₅) Potassium (K ₂ O)	3000-6000 2400-3500 ~ 60,000	3000-6000 ~ 600 -	250-350 70-140 40-60	45-60 45-60 60-100	

Speaking of soils as systems saturated with microorganisms, one must note that they usually are rather rich in the elements required for the nutrition of crop plants. However, most of these compounds are in a form unavailable or almost unavailable to the plant. Table 1 gives data on the total content of nitrogen, phosphorus, and potassium in one hectare of plowed podzolic soil. These figures are comparable to the corresponding plasma reserves of live bacteria inhabiting the soil, and to the usual average proportions of inorganic fertilizers. Obviously, these calculations are very rough.

As shown by these figures, the total content of nutrients in the soil is huge. By comparison, the quantities of such elements introduced by fertilizers is extremely small. Nevertheless, fertilizers have their use, since the /168 seedlings are nourished by substances that are easily assimilated by plants. At the same time, it is a fact that the principal reserves of the soil do not remain dead capital forever. Under bacterial action, compounds only slightly accessible to plants may be converted into substances easily assimilated by the crop.

The nutrient reserve of the soil is also built up by bacteria, from elements present in the air. Thus nitrogen-fixing bacteria may fix from 50 to 250 kg of nitrogen (sometimes even more) per hectare of soil during a single vegetal cycle (D.N.Pryanishinkov).

It is obvious that the bacterial population of the soil, under favorable circumstances, is readily able to absorb a greater or smaller proportion of the nutrients introduced into the soil, thus complicating their supply to the plant.

It must be remembered that microorganisms are not uniformly distributed in soils. They live in particularly large colonies around the roots of plants, in the zone of the so-called rhizosphere. As N.A.Krasil'nikov correctly pointed out, the microflora of the rhizosphere of a plant is highly important to plant life. Primarily, it constitutes a kind of filter through which pass the nutrient elements, where they are, to a certain extent, modified and absorbed by microorganisms. At the same time, the bacteria produce several organic compounds of vital importance to the plant (of the type of vitamins and growth factors) which are assimilated by the root system.

Thus the activity of soil microorganisms is closely linked to the nutrition and growth of agricultural plants. This fact must not be ignored, as has been done by some scientists, like Pabst. Under the influence of these authors, some experts have wrongly come to think that soil productivity depends only on the degree to which the nutrients removed by the crop are returned to the soil. The proponents of this view recommended a total return to the soil of any substance removed from it by the harvested plants, essentially viewed the soil as a dead substrate, and attempted to regulate its fertility on the basis of an arithmetic balancing of outflow and inflow of soil nutrients. Our own agrochemists have substantially revised these concepts.

In criticizing these views, Vil†yams wrote that to insist on this view-point after the outstanding work of numerous microbiologists and biochemists (Pasteur, Bakh, Vinogradskiy, Kholodniy, and others) would mean to be almost half a century behind in development.

In solving the problem of plant nutrition, the microbiologist is faced by the following tasks:

- a) find ways and means to activate, even if only partially, the soil reserves inaccessible to plants;
- b) participate in the development of the most effective methods of fertilizing agricultural crops;
- c) rationalize the application of bacterized fertilizers.

This raises the question as to the methods, making use of the activity of microorganisms, for converting the potential fertility of the soil into actual fertility. The farmer usually restricts himself to a certain system of cultivation for activating the mobilization processes. Such a method is still largely empirical, it is not based on microbiological proofs, and it is not necessarily effective, although it is of some use. However, the microbiological basis of /169 various agricultural engineering methods for influencing the soil must be worked

out in more detail (see below).

It is well known that microorganisms are able to degrade compounds that cannot be decomposed by the most effective chemical means. Substances such as granite, silicates, mica, etc. are susceptible to microbiological erosion. On this basis, it has been suggested to inoculate the soil with bacterial agents capable of converting complex compounds into forms available to the plants. These include preparations of phosphobacteria capable of mineralizing organic phosphates; of silicate-decomposing bacteria; of AMB for activating soil biodynamics; etc.

The use of these preparations has some effect, but it is plainly insufficient and unstable. To obtain the action of desirable bacteria in the soil requires corresponding modifications of the substrate in which the microorganisms must exist. This fact is frequently forgotten, and bacterial preparations are handled like chemical fertilizers. This leads to inevitable disappointment.

Further data are required on the mode of action of bacteria that mobilize the low-mobility compounds of the soil. This will permit an active intervention in the life of the soil and a modification of its reproductive power.

It can be stated that some farm experts object, in principle, to measures that put into the cycle compounds otherwise fixed in the soil. They deprecate such measures as a waste of the valuable reserves of the soil. Such fears seem exaggerated, since a rational utilization of the potentialities of the land must be combined with the careful preservation of its agronomically useful properties.

Next, we will discuss the problem of effectively fertilizing the soil with mineral substances. Fertilizer must be used such as to meet the requirements of agricultural crops to the greatest extent. Vil'yams always emphasized: "I am against fertilizing the soil; I fight instead for fertilizing the plants" (Bibl.5, p.130).

This proposition is correct in principle; however, Vil'yams committed the error of considering that inorganic fertilizers were unprofitable on nonstructured soils, and therefore should not be used. This led to a refusal to fertilize crops, in a number of localities of the USSR.

T.D.Lysenko pointed out that, on the basis of the Vil'yams' theory of the rise and fall of soil fertility, methods of applying inorganic fertilizers can be adjusted to increasing the effectiveness of the fertilizer without doing damage to the soil structure. An example of the use of pelleted fertilizers shows that high productivity can be obtained with only small portions of fertilizers.

There is practically no rule for the technique of applying inorganic fertilizers from the microbiological viewpoint, so that we can only give a few theoretical aspects. It should be noted that the introduction of mineral matter sharply increases the growth of various microorganisms in the soil. This is obvious from the data in Table 2, obtained in one of our experiments.

On the basis of these data, it may be asserted that the effect of inorganic

fertilizers cannot be explained by their chemical nature alone. The application of fertilizers has a marked effect on the entire soil biodynamics which, in /170 turn, affects the development of the crop.

TABLE 2
EFFECT OF FERTILIZERS ON SOIL MICROFLORA

Fertilizer		Number of Microo Thousands per Gra Soil	Percent of Soil		
	Bacteria	Actinomycetes	Fungi	Granules with Cellulosolytic Microorganisms	
Control Phosphorus-potash	1120	760	11	10.5	
mixture Phosphorus-nitrogen-	2040	1030	25	69.0	
potash mixture	3850	1350	24	72.0	

It seems obvious that the type of inorganic and of organo-inorganic fertilizers, as well as the technique and time of their application, cannot be established without considering the activity of soil microorganisms. The application of these questions of microbiology to practical farming has only just begun.

In connection with the above questions, it is necessary to determine how soon after the death of bacterial cells their substances are mineralized.

In his fertilizer system, Vil'yams paid special attention to the use of farm manure, and also to the combination of organic and inorganic fertilizers. One cannot, however, agree with his view that only rotted pulverized manure should be applied in grassland crop rotation, since manure has no substantial effect on the establishment and maintenance of soil fertility conditions. At the same time it is generally known from agricultural practice that in the non-arid zone, the application of manure on fallow fields is more useful than the conversion of the same amount of manure into humus for subsequent application to the soil (Lysenko).

Speaking of the fate of inorganic fertilizers in the soil, one must remember not only their biological fixation but also their chemical fixation. The latter extremely undesirable phenomenon must be controlled by suitable methods of applying fertilizer.

In the near future, more attention must be paid to bacterial fertilizers. Two forms of such preparations are widely used today, namely, nitragin and azotogen (azotobacters).

In many cases these agents, especially the nitragin, have a good effect and markedly improve the harvest. There are also many ambiguities in their use,

which should be eliminated.

Up to now the existence of ecological and geographical strains among the various genera of bacteria has been disregarded in the formulation of bacterial fertilizers. Over the entire vast territory of the USSR, the very same type cultures of nitrogen-fixing bacteria are being used. This is definitely wrong.

It will be necessary to manufacture bacterial agents of specific breeds of nitrogen-fixing bacteria and, in particular, to expand the use of local bacterized fertilizers. The production of the latter may be based on various methods; some of these have been recommended to the Ministry of Agriculture USSR by the Institute of Microbiology, Academy of Sciences USSR, as far back as 1941.

In the manufacture of bacterized fertilizers from pure cultures, emphasis must be placed on maintaining their activity, for which it is desirable to /171 work out improved methods of cultivating bacteria that fix gaseous nitrogen.

It must be considered unfortunate that no clear definition has ever been made as to the category of soils in which bacterized fertilizers are highly effective. Particularly unsuitable is the use of azotogen. It is well known that nitrogen-fixing bacteria are highly particular as to their substrate. They develop well only in neutral soils, relatively rich in calcium, phosphorus, and potassium. These bacteria are also hydrophilic. Studies in our Laboratory have shown that they require at least twice as much moisture as other saprophytic soil bacteria.

It is therefore not surprising that azotobacters, introduced into soils of unwatered crops in semi-arid zones will die out rather rapidly.

Facts similar to these have even led certain investigators (N.N.Sushkina) to the conclusion that azotobacters are unable to grow in the chernozem belt, in chestnut and more southern soils, since the steppe vegetation has a "fatal" effect on them.

According to Vil'yams, all annual field crops belong to the steppe formation. For this reason and based on Sushkina's proposition, it must be concluded that azotogen should not be used here at all. This conclusion is in itself questionable, and we consider it unjustified. According to our own observations, nitrogen-fixing bacteria develop in the steppe regions as short-lived organisms during the period of adequate moisture in the soil; consequently, azotogen may be used, but only with proper consideration of the moisture conditions of the soil. This example demonstrates that more attention should be paid at present to discussing the question of what type of soils and crops should be given azotogen.

As already noted, the use of nitragin must also be further specified. Large amounts of bacterized fertilizers are being used under conditions where no significant effect can be expected.

In many cases, the use of bacterial agents, in combination with inorganic and organic fertilizers, should be thoroughly investigated. One cannot forget

the words of Vil'yams: "Grassland farming demands simultaneous improvement of plant nutrition and feeding of the bacterial population, for which reason I am in favor of a combination of inorganic and organic fertilizers" (Bibl.5, p.146).

We do know that leguminous plants are best grown on organic fertilizers, including those with straw content. Certain inorganic fertilizers (especially phosphorus-containing types), used simultaneously with nitragin on leguminous crops, have a similar excellent effect.

The question of the mycotrophic nutrition of crops is an unexplored field of agricultural microbiology. Vil'yams was much concerned with this aspect, believing that the phenomenon of symbiosis must be of equal importance here as in woody vegetation. It is well possible that, in many cases, the decreased yield of crops can be explained by a disturbance of the symbiotic association of higher plants and fungi.

Activity of Microorganisms in Connection with Soil Cultivation and Irrigation

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Vil†yams developed a theoretical basis for soil cultivation. His system of cultivation is of major scientific and productive significance. In this system, the cultural plowing of soil is done primarily by plows with colter. Such plowing must be done to a depth of not less than 20 cm.

Vil'yams placed considerable emphasis on the proper time and technical procedure of soil cultivation. Plowing and other operations on the soil that affect the air-water regime sharply modify the microbiological processes at the cultural horizon.

We cannot discuss here the well-known details of the Vil'yams doctrine on the cultivation of the upper layer and the cultural plowing of the soil. We note merely that Vil'yams not only viewed soil cultivation as an operation to improve aeration and water conditions in the plowed layer, but he also attached great importance to the displacement of the soil layers. For example, in a cultured soil, the rotation of the upper layer to the bottom of the furrow creates relatively anaerobic conditions for the microflora. This leads to the formation of humic substances of the type of ulminic acid and to a partial improvement of the soil structure.

The lowest layer of soil, richer in readily mineralized organic matter, when shifted to the top and when aerobic processes are able to develop in it, will furnish a large part of the nutrients required by the plants.

These examples demonstrate clearly that the plowed layer is not uniform and that, in its individual parts, the processes are far from identical. However, this is usually forgotten, and the biodynamics of the plowed layer of the soil is characterized by average data, masking the factors that determine the size of the crop.

A typical example is given to illustrate the biogenicity of the individual layers of the plowed soil (see Table 3). The data are taken from one of our

studies in an experimental field of the Timiryazev Agricultural Academy. Of course, a different picture might be obtained on other types of soil. Thus, it is clear from Table 3 that the largest part of the viable bacteria populates the uppermost layer of the podzolic soil. In more southern regions, this layer dries out in summer, so that microorganisms are unable to develop full activity.

TABLE 3

DISTRIBUTION OF MICROORGANISMS IN VARIOUS LAYERS OF PODZOLIC SOIL

Layer of Soil, in cm	Total Number of Bacteria, in Thousands per Gram of Soil	Percentage of S Giving Gro	Number of Fungi, in	
		of Nitrogen- Fixing Bacteria	of Cellulose Bacteria	Thousands per Gram of Soil
0 - 5 10 - 15 15 - 20	3600 1500 1200	15 10 6	6 2 0	15 6 1

These remarks permit the assumption that a scientific theory of soil cultivation cannot be established without taking account of the activity of lower organisms in the various parts of the soil layer occupied by the roots.

Obviously, no single method of soil cultivation can be given for all zones of the USSR. The details must be determined at the individual locations, starting from the general theoretical principles developed by Vil'yams.

In the near future, the desire for high yields will lead to a marked expansion of existing irrigation systems in the south of the USSR and to the irrigation of large areas in the regions of insufficient rainfall in the chernozem belt. The water regime of the soil and the activity of soil microorganisms has been studied by many investigators, but always under laboratory conditions. The results are difficult to apply to the situation in the field, and it is obvious that they must be put into a more concrete form. The data obtained, together /173 with results of plants physiology, will help in solving the problem of correct irrigation schedules.

The problem of the cultivation of peat soils occupies a special but very important position for many localities of the USSR. In this case, the problem is not limited to the drainage of excess water. One must also gradually convert the difficultly mineralized organic matter of the peat into compounds that can be assimilated by the agricultural crops. Peat soils to be reclaimed contain an almost inactive microflora, and it may well be that their bacterization, at simultaneous agricultural engineering and agricultural chemical work, will greatly promote increased productivity.

Microbiological Methods for Soil Testing

In characterizing the type of soil and determining its state, microbiological indices are used together with others. The basic premise here is the correct assumption that the lower organisms are the most exact indicators and often can be used more successfully in defining the properties of a given soil than the chemical methods.

Until recently, however, the fundamental principles of microbiological soil testing were poorly developed. Earlier, the quality of a given soil was evaluated on the basis of a count of the total number of microorganisms or of the number of species of various physiological groups of such microorganisms. Analyses of this type do not yield sufficiently accurate results, since they disregard the behavior of the individual species of microorganisms in the soil. Yet it is only by determining the state of the latter, instead of the changes in the groups of bacteria consisting of a substantial number of representatives of the lowest organisms, that an accurate idea can be obtained of soil properties of interest to soil scientists and agronomists.

The Institute of Microbiology, USSR Academy of Sciences, has recently developed new methods for microbiological soil testing, also taking account of the results of earlier work. Of course, the methods are not complete with respect to methodological questions, which must be further investigated.

Nevertheless, we believe that our analytical principle, based on the species composition of the microflora, will be more satisfactory in domestic agricultural practice than the older testing methods. The approach to such testing /174 was discussed by us in the article "The Dokuchayev-Kostychev-Vil'yams Soil Doctrine and the Question of the Microflora Composition in Phytogenesis", to which we refer the reader who desires more detailed information on this problem.

It seems that the methods of determining the mobile nutrients in the soil developed by Soviet microbiologists (Butkevich, Kryuchkova, and others) have been forgotten in the meantime and are no longer in use. Measures should be taken for their re-introduction into laboratory practice.

Microbiologists should not disregard methodological questions. Their solution largely determines the possibility of using microbiological methods in practical agriculture.

Our survey cannot be considered exhaustive. Nevertheless, it demonstrates the great significance of microorganisms for increasing the yield, and the correctness of Karl Marx' statement: "With the development of natural sciences and agronomy the fertility of the soil will change, since the means for a direct utilization of the soil elements also undergo changes" (Bibl.44).

In the USSR, the progressive workers of agriculture obtain yields unheard of elsewhere in the world. They are radically changing the basic nature of the processes in the soil. Scientific workers, including microbiologists, should base the solution of the problem of improving fertility on the experience of these farm experts.

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